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Lesion recovery and the bacterial microbiome in two Caribbean gorgonian corals

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Abstract In the Caribbean, gorgonian corals dominate many coral reefs, while scleractinian coral cover has declined. Gorgonian corals deal with stressors such as lesions caused by wave action, predation, or human activities. In June 2012, artificial wounds were inflicted on branches of the gorgonians *Eunicea flexuosa* and *Pseudoplexaura porosa* found at 3–5 m depth on a patch reef (20°52′5.23″N, 86°51′58.92″W) near Puerto Morelos, Mexico. Following healing, injured and uninjured branches were collected to determine the effect of lesions on gorgonian biochemistry, symbiosis, microbiome, and immune response. Lesion recovery in *E. flexuosa* took twice as long as in *P. porosa*. In both species, tissues at and surrounding the lesions contained significantly higher sclerite content per dry weight but lower protein per surface area. In and around the lesion area, the density of symbiotic dinoflagellates, *Symbiodinium* spp., was lower than in uninjured branches, although *Symbiodinium* photochemical efficiency in tissues surrounding the lesion was not affected. The gorgonian species differed in their bacterial microbiome, but the overall bacterial community and dominant bacterial taxa did not differ between injured and uninjured branches, although the prevalence of some less common bacterial groups did vary. The two species exhibited distinct immune responses, whereby different components of the melanization cascade were activated, and exochitinase was mobilized only in *E. flexuosa*. While the gorgonian species differed in their lesion recovery response, both healed without signs of disease or colonization by fouling organisms. The capacity to recover successfully from injuries may partly explain why gorgonian corals dominate Caribbean coral reefs.

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Introduction

In contrast to scleractinian corals whose abundance on Caribbean reefs has dramatically declined over the past few decades (Gardner et al. 2003; Alvarez-Filip et al. 2011; Jackson et al. 2014), gorgonian corals dominate many Caribbean reefs (Goldberg 1973; Kinzie III 1973; Jordán-Dahlgren 1989; Ruzicka et al. 2013; Villamizar et al. 2014; Lenz et al. 2015). They often serve as shelter and a food source for coral reef organisms (Voss 1956; Birkeland and Neudecker 1981; Lasker et al. 1988; Vreeland and Lasker 1989). Despite their dominance, knowledge about gorgonian physiology (e.g., Cary 1918; Kanwisher and Wainwright 1967; Lewis and Post 1982; Ramsby et al. 2014; Shirur et al. 2014) and their microbial consortia (Toledo-Hernández et al. 2008; Sunagawa et al. 2010; Hewson et al. 2011; Duque-Alarcón et al. 2012; Correa et al. 2013; Tracy et al. 2015; McCauley et al. 2016; Robertson et al. 2016) is scant. This paucity of data applies both to ambient and adverse conditions, such as injury, that may compromise gorgonian colonies. Investigating lesion healing in gorgonian corals is one-step toward understanding gorgonian abundance on Caribbean reefs.

Due to the upright arborescent morphology of many gorgonian species and their soft tissue, they can be damaged by wave action such as during storm surges (Cary 1914; Wahle 1985) or entanglement with fishing gear (Chiappone et al. 2005). Injuries can range from mild abrasion to uprooting of a colony and result in partial or whole colony mortality. Predation by butterflyfish, *Chaetodon* spp. (Birkeland and Neudecker 1981; Lasker 1985), the polychaete, *Hermodice* sp. (Vreeland and Lasker 1989), and snails, *Cyphoma* spp. (Lasker et al. 1988; Ruesink and Harvell 1990) also leads to lesions in gorgonian corals. While the butterflyfish feed exclusively on polyps, the worms and snails feed on multiple gorgonian tissues. In turn, pathogens may colonize the wound. The injury site may also serve as a substrate for other species, as in the case of the fire coral *Millepora* sp., which can overgrow parts or entire colonies of the gorgonian *Plexaura homomalla* (Wahle 1980; Gerhart 1990).

Growth of new tissue at a lesion site requires organic molecules. In scleractinian corals, these molecules may migrate from adjacent uninjured tissues or other parts of the colony (Oren et al. 2001; Henry and Hart 2005). In gorgonian corals, food particles and organic molecules can be transported within branches (Murdock 1978a, b), but the effects of lesions on the biochemical composition of injured and uninjured branches have not been assessed. Since in gorgonian species, the amount of non-skeletal material (soluble protein, lipids and carbohydrates) per dry weight varies from 4 to 25 % (Shirur et al. 2014), lesion recovery may differ between species if recovery is correlated to tissue content. Furthermore, similar to scleractinian corals, many gorgonian species host endosymbiotic dinoflagellates, *Symbiodinium* spp. Lesions may affect *Symbiodinium* photosynthesis or density in gorgonian cells around the lesion site.

Lesions on gorgonian branches may enable pathogens to colonize the injury site. Under ambient, non-stressful conditions, various bacteria, viruses, fungi, and protists inhabit tissues of scleractinian and gorgonian corals (Knowlton and Rohwer 2003; Toledo-Hernández et al. 2008; Sunagawa et al. 2010; Hewson et al. 2011; Burge et al. 2012; Duque-Alarcón et al. 2012; Correa et al. 2013; McCauley et al. 2016; Robertson et al. 2016). In scleractinian corals, bacteria are important in cycling sulfur and nitrogen, and some bacteria may limit the growth of opportunistic taxa (reviewed in Thompson et al. 2015). Diseased tissues of scleractinian and octocorals exhibit a different bacterial community than healthy tissues (Sunagawa et al. 2009; Cárdenas et al. 2012; Vezzulli et al. 2013; Meyer et al. 2014; Roder et al. 2014).

Any biological changes at the injury site may trigger an immune response. Gorgonian corals produce a suite of compounds with antimicrobial and antifungal activity (Perkins and Ciereszko 1973; Rodríguez 1995; Jensen et al. 1996; Kim et al. 2000; Hunt et al. 2012). In addition, amoebocytes in gorgonian corals can phagocytize foreign particles (Olano and Bigger 2000; Ruiz-Diaz et al. 2013) and prevent the entry, or limit proliferation of pathogens by depositing melanin (Petes et al. 2003; Mydlarz et al. 2008). During lesion recovery in several scleractinian corals (D'Angelo et al. 2012; van de Water et al. 2015), and when the gorgonian coral *G. ventalina* was exposed to fungal pathogens (Mydlarz et al. 2008), the activity of phenoloxidase (PO), the key enzyme of the melanization cascade (Cerenius and Söderhäll 2004), increased. Invading organisms may also be killed by cytotoxic intermediates of the pathway like quinones and reactive oxygen species (Cerenius and Söderhäll 2004). In addition to these general immune responses, fungal pathogens are inhibited by the antioxidant peroxidase (POX) and protease inhibitors (Mydlarz and Harvell 2007; Mann et al. 2014), and the release of exochitinase (EXOC) into the surrounding water (Douglas et al. 2007). In gorgonian corals, it is unknown how the immune defenses react to the occurrence of a lesion. The current study tested the hypothesis whether Caribbean gorgonian branches inflicted with lesions differed from uninjured branches in their biochemical composition, *Symbiodinium* parameters, microbiome, and immune response.

Methods

Study site and experimental design

This study occurred in June 2012 on a patch reef (20°52′5.23″N, 86°51′58.92″W) near the Instituto Ciencas del Mar y Limnologia in Puerto Morelos, Mexico. At 3–5 m depth, eight colonies each of the gorgonian corals *Eunicea flexuosa* and *Pseudoplexaura porosa* were tagged. A branch in each colony was chosen, and to simulate injury, 2 cm below the apical tip, the cortex (the outer tissue region housing the polyps and embedded with sclerites) was removed from one side, exposing the underlying proteinaceous axial rod (Fig. 1a, e). The resulting lesion was 10 cm long and approximately half the width of the branch. A branch in a different area of the same colony was chosen as an uninjured control.

Photochemistry of photosystem II (PSII)

Using a fluorometer (Diving-PAM, Walz, Germany), the maximum (at local dusk: F_v/F_m) and effective (at local noon: $\Delta F/F_{m'}$) photochemical efficiency of PSII was recorded every 2 days. Photochemical efficiency was measured on the injured branch in tissue adjacent to the top, middle, and lower area of the lesion, and in corresponding

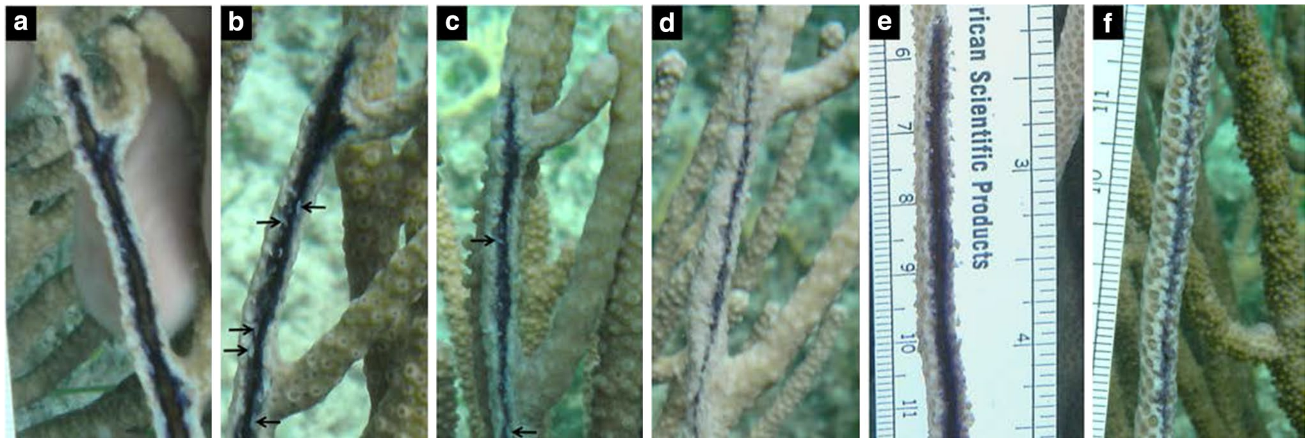

Fig. 1 Lesion recovery in photographs of representative branches of the gorgonian corals *Eunicea flexuosa* (**a–d**) and *Pseudoplexaura porosa* (**e**, **f**) following artificial injury. The lesion on day 1 (**a**, **e**), 4 (**b**), 7 (**c**, **f**) and 14 (**d**) days post injury. *Black arrows* in (**b**) and (**c**) point to sclerites visible along the lesion perimeter of *E. flexuosa*

regions of the uninjured control branch. From the effective and maximum photochemical efficiencies, the maximum excitation pressure over PSII (Q_m) was calculated (Iglesias-Prieto et al. 2004).

Sample collection and processing

Gorgonian branches were collected when, at the lesion site, tissues were flush with surrounding uninjured tissues, and newly regenerated polyps emerged along the perimeter (Fig. 1d, f). This occurred after 7 days in *P. porosa* and 14 days in *E. flexuosa*. Measuring from the branch tip, 20-cm-long fragments were cut from both the injured and uninjured branches. These fragments were sub-partitioned, and 2-cm pieces, located 6–8 cm from the branch tips, were removed. The length, diameter, and wet weight of the 2-cm pieces were recorded, and both the 2-cm pieces and the remaining branch segments were frozen in liquid nitrogen and stored at −80 °C until further processing.

Symbiodinium parameters and host protein were quantified from the 2-cm-long pieces. The remaining branch segments were processed for the other assays by separating the cortex from the inner axial rod and discarding the rod. For the injured branch, subsamples were taken from regenerated tissue at the lesion and uninjured tissue on the other side of the branch (8–11 cm from the branch tip), and from tissue at least 2 cm below the lesion (14–15.5 cm from the branch tip). For the uninjured branch, subsamples were taken from 8 to 9.5 cm from the branch tip. Subsamples were freeze-dried prior to analysis of sclerite, protein, carbohydrate, and melanin content.

Determination of *Symbiodinium* cell density and chlorophyll *a* (Chl *a*) and c_2 (Chl c_2) content followed described protocols (Shirur et al. 2014), standardizing the values per surface area, per mg host protein or per symbiont cell. *Symbiodinium* cells were enumerated using a FlowCAM particle analyzer (Fluid Imaging Technologies Inc., USA). Host protein was quantified from the supernatant in the *Symbiodinium* isolation protocol (Dove et al. 2006). Sclerite, protein, and carbohydrate content determination followed the protocols described in Shirur et al. (2014), with the contents standardized to dry weight of the cortex (%g DW) (Shirur et al. 2014).

Genetic identification of *Symbiodinium* and bacteria

DNA was extracted from regenerated tissue at the lesion and the uninjured tissue adjacent to it (on the other side of the branch), and in the uninjured branch from tissues located roughly 4 cm away from the branch tip, following standard protocols (Shirur et al. 2014). The internal transcribed spacer 2 region (ITS2) of the ribosomal DNA was used for *Symbiodinium* identification (Shirur et al. 2014).

The bacterial microbiome of gorgonian tissues was characterized based on a 250-bp portion of the bacterial 16S rRNA gene using Illumina MiSeq sequencing (Kozich et al. 2013). Samples were pooled, spiked with 5 % PhiX (Jackson et al. 2015), and sequenced at the University of Mississippi Medical Center Molecular and Genomics Core Facility. Raw sequences were processed using the bioinformatics software mothur 1.35.1 (Schloss et al. 2009, 2011) according to the protocol of Kozich et al. (2013). Sequences were aligned against the SILVA 16S rRNA database (Quast et al. 2013), and chimeras detected using UCHIME (Edgar et al. 2011). Valid sequences were classified using the Greengenes 16S rRNA classification scheme (DeSantis et al. 2006). Archaeal and eukaryotic sequences were removed, and the bacterial sequences were grouped

into operational taxonomic units (OTUs) based on 97 % sequence similarity.

Melanin content and enzyme activity

Melanin was extracted from the freeze-dried tissues following the Palmer et al. (2011a) protocol and standardized to the amount of organic matter within the sample (Shirur et al. 2014). For the injured branch, enzyme activities were quantified from regenerated tissue at the lesion and uninjured tissue adjacent to the lesion (on the other side of the branch, both 3–6 cm from the branch tip) and from tissues at least 2 cm below the lesion (17–18.5 cm from the branch tip). To quantify enzyme activity, proteins were extracted in 100 mM phosphate buffer pH 7.8 using the Mydlarz and Harvell (2007) protocol.

Total potential phenoloxidase (PO) activity was quantified using the Palmer et al. (2011a) protocol. Peroxidase (POX) activity was obtained by modifying the Mydlarz and Harvell (2007) protocol whereby 20 µl of the extract was diluted with 20 µl of 0.01 mM PBS pH 6.0, and 25 µl of 25 mM guaiacol was added to the dilution. The reaction was initiated with 60 µl of 25 mM H_2O_2. For PO and POX, the change in absorbance per minute was calculated from the linear portion of the curve. Exochitinase (EXOC) activity was estimated by modifying the Couch et al. (2008) protocol using 60 µl of the diluted extract in the assay. Background fluorescence was quantified in wells containing either the sample or substrate and aliquots of sodium acetate buffer and 0.5 M Na_2CO_3, and it was subtracted from fluorescence detected in the samples. Enzyme activity assays were run in duplicate and normalized to mg protein present in the aliquot of extract used. Protein content (mg ml^{-1}) of the enzyme extract was determined using the RED660™ Protein Assay Kit (G-Biosciences, USA). PO and EXOC activity were quantified in both species, but POX activity was only measured in *E. flexuosa* because the mucus in *P. porosa* interfered with the assay.

Statistical analyses

With the exception of the bacterial community analyses, the two gorgonian species were analyzed individually since the time to lesion closure differed between them. For most comparisons, linear mixed-effects models using the restricted maximum likelihood method were run with the lme4 (Bates et al. 2013) and lmerTest (Kuznetsova et al. 2014) packages within R 3.2.2 (R Core Team 2015). Photochemistry was analyzed with a linear mixed-effects model where treatment (tissue adjacent to the lesion and uninjured branches) and time (days) were the fixed effects and parent colonies were the random effect. A paired Student's *t* test was used to test differences in host protein and the *Symbiodinium* parameters between tissues from the wounded region of the injured branch (which included regenerated tissues at the lesion and uninjured tissue on the other side of the branch) and those from the uninjured branch.

In the linear mixed model used to assess the lesion effect on tissue biochemical composition, melanin content, and enzyme activities, the fixed effect was the experimental treatment (four levels: regenerated tissue at the lesion, uninjured tissue adjacent to it on the other side of the branch, and at least 2 cm below the lesion, and tissue from the uninjured branch), and the parent colony was the random effect. Results of the mixed model analyses were further explored using post hoc planned contrasts with the multcomp package (Hothorn et al. 2008). For each parameter, values at the lesion were compared to those adjacent to it. When significant differences occurred, the tissues adjacent to the lesion were compared to those below it (i.e., uninjured at the lesion area vs. uninjured below lesion area). If the injured and adjacent tissues were not statistically different, they were pooled and compared to tissues sampled from below the lesion. If the three regions in the injured branches did not significantly differ from each other, they were pooled and compared to tissues of the uninjured branch. If tissues at and adjacent to the lesion significantly differed from tissues below it, then the latter was separately compared to tissues from the uninjured branch. The *P* values for all comparisons were corrected using the Bonferroni method (Westfall 1997).

Bacterial community structure was explored with the theta index of dissimilarity (Yue and Clayton 2005), using mothur 1.35.1. To standardize for different sequence depth between samples, data were randomly subsampled to 425 sequences per sample (the lowest number of valid reads from any sample), and the mean of 1000 subsampling iterations was used for all analyses (Schloss et al. 2011). Analysis of molecular variance (AMOVA) was run on the whole dataset to test whether the bacterial community differed between *E. flexuosa* and *P. porosa*, and again for each gorgonian species separately to test whether the community differed between injured and uninjured tissues. Indicator analysis identified bacterial OTUs responsible for the detected differences. Linear mixed-effects models tested the effect of injury on the relative abundance of bacterial taxa (phyla, families, and individual OTUs) in tissues of each gorgonian species, where treatment (three levels: regenerated tissue at the lesion, uninjured tissue adjacent to it on the other side of the branch, and tissue from the uninjured branch) was the fixed effect, and parent colonies were the random effect. To test the effect of injury on the number of OTUs found in tissues, a generalized linear model (using a Poisson distribution) was used.

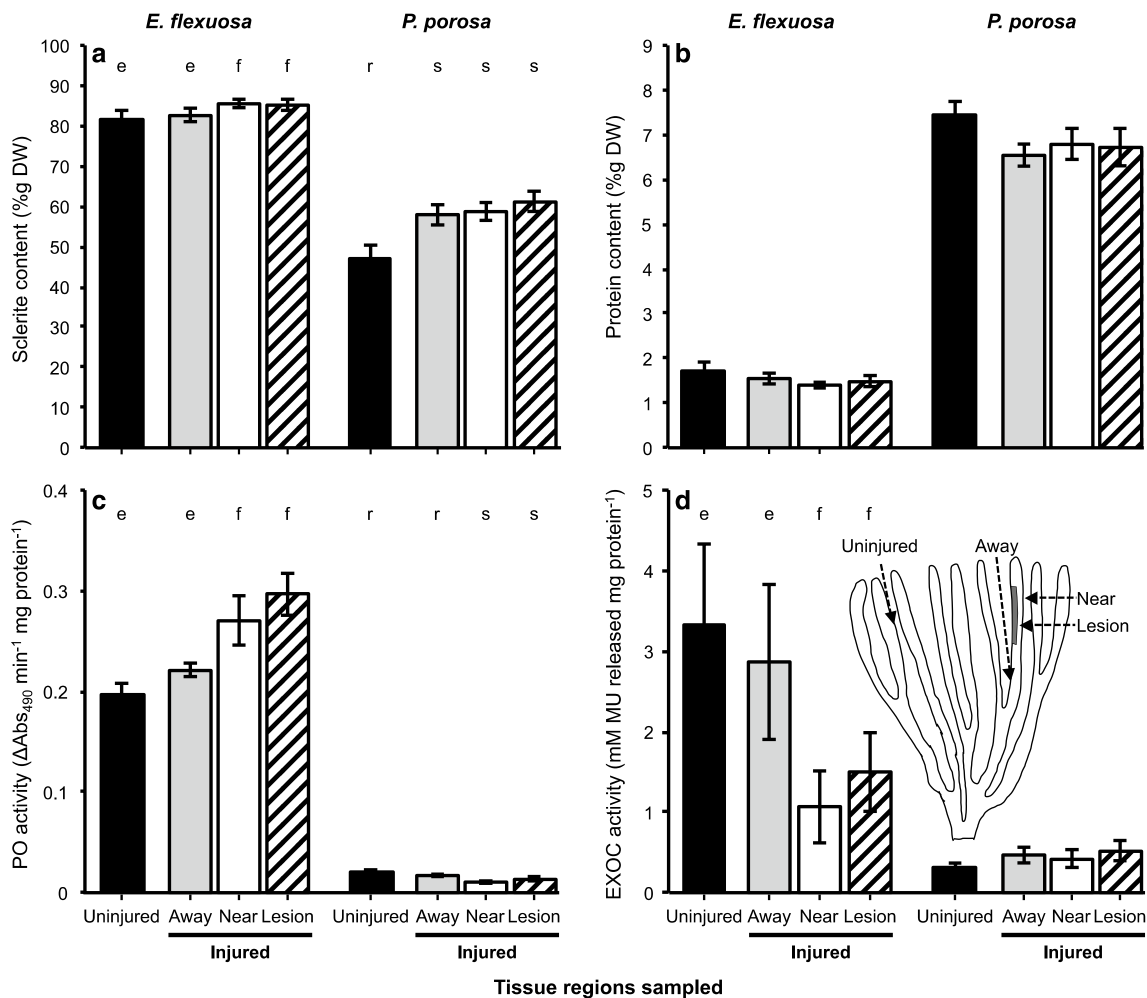

Fig. 2 Mean **a** sclerite and **b** protein contents per dry weight (%g DW), and activities of the enzymes, **c** total potential prophenoloxidase (PO) and **d** exochitinase (EXOC) in tissues of the gorgonian corals *Eunicea flexuosa* and *Pseudoplexaura porosa*. Samples (see inset) were taken from uninjured branches (Uninjured) and three regions of the injured branches (Injured): branch tissue away from the lesion (Away), uninjured tissue adjacent to the lesion (Near), and regenerated tissue at the lesion (Lesion). Data are mean ± SE with $n = 6$ for most data and $n = 5$ for *P. porosa*-Uninjured. *Letters* (*e*, *f* for *E. flexuosa* and *r*, *s* for *P. porosa*) above the *bars* denote significant differences ($P < 0.05$) in the planned contrasts

Results

Lesion recovery

The artificial injury exposed the axial rod to the environment (Fig. 1a, e). In both gorgonian species, 4 days post injury, the axial rod was no longer visible, and tissues at the lesion were violet in contrast to the beige coloration of adjacent uninjured tissues and the rest of the colony. The healing process, however, differed between the two gorgonian species. In *E. flexuosa,* large violet and white spindle-shaped sclerites were visible in the wound (Fig. 1b, c). These sclerites were subsequently overgrown by tissues (Fig. 1c, d). A week post injury in *E. flexuosa,* the deep gash in the center of the lesion still remained, and tissue along its perimeter was devoid of polyps (Fig. 1c). The gradual advance of opposing fronts until they fused with each other, and the formation of polyps along the lesion perimeter, took an additional 7 days (Fig. 1d). Conversely, in *P. porosa*, surface tissues and polyps occurred at the lesion site a week post injury (Fig. 1f), and small dark pigmented sclerites were visible underneath the tissues.

Biochemical composition of tissues

In *E. flexuosa*, the sclerite content of the cortex in tissues at and surrounding the lesions was significantly greater than in tissues below the lesions and in the uninjured branches [Fig. 2a, ANOVA, $F(3, 15) = 4.0$, $P = 0.029$]. In contrast, sclerite content in *P. porosa* did not differ between

Table 1 Effect of injury on parameters in the gorgonian corals *Eunicea flexuosa* and *Pseudoplexaura porosa*

Parameter	Uninjured	Injured			$F(\mu, \nu)$	P
		Away	Near	Lesion		
E. flexuosa						
Host protein (mg cm^2)	$0.42^{!} \pm 0.06$	NA	$0.23^{!} \pm 0.02$		$t6 = 3.56$, $P =$ **0.012**	
Carb %g DW	0.73 ± 0.06	0.79 ± 0.06	0.84 ± 0.14	0.66 ± 0.07	$F(3,15) = 1.3$	0.305
Melanin %g OM	$1.37^{\#} \pm 0.12$	$1.29^{e} \pm 0.07$	$1.19^{e} \pm 0.11$	$0.95^{f} \pm 0.09$	$F(2, 10) = 7.4$	**0.011**
POX$^{\wedge}$ activity	$0.24^{e} \pm 0.07$	$0.23^{\#e} \pm 0.05$	$0.34^{f} \pm 0.06$	$0.57^{f} \pm 0.15$	$F(3,14.2) = 3.8$	**0.034**
P. porosa						
Host protein (mg cm^2)	3.24 ± 0.36	NA	1.96 ± 0.23		$t5 = 3.25$, $P =$ **0.023**	
Carb %g DW	$2.83^{\$} \pm 0.25$	$2.10^{\#} \pm 0.10$	$2.44^{\#} \pm 0.21$	$2.53^{\#} \pm 0.31$	$F(3, 11.3) = 1.6$	0.249
Melanin %g OM	$1.02^{\#} \pm 0.03$	1.26 ± 0.13	1.17 ± 0.05	1.07 ± 0.12	$F(2, 10) = 1.3$	0.319

Samples were processed from the uninjured branch (Uninjured) and three regions in the injured branch (Injured): tissue at least 2 cm below the lesion (Away), tissue adjacent to the lesion on the other side of the branch (Near), and regenerated tissue at the lesion (Lesion). Host protein content was not obtained from the away area. Melanin content was only tested within sampled regions of the injured branches. Values are mean ± SE, with $n = 6$, $n = 7$ (!), $n = 5$ (#), or $n = 4$ ($). *DW* dry weight, *OM* organic matter, Carb = carbohydrate, POX = peroxidase, $F(\mu, \nu)$ = *F*-ratio and degrees of freedom, *P* = probability of the null, <0.05 in bold. Differences in host protein were tested using paired *t* tests, where *t* = test statistic. To meet normality and heteroscedasticity assumptions, some parameters were log-transformed (^). Superscript letters (e, f for *E. flexuosa*, r, s for *P. porosa*) denote significant differences ($P < 0.05$) in the planned contrasts

the three regions of the injured branches, but was significantly higher in the injured than in the uninjured branches [Fig. 2a, ANOVA, $F(3, 14) = 24.9$, $P < 0.001$]. The mean protein content of the cortex did not differ between the four sampled regions (injured branch: injured, adjacent uninjured and below uninjured, and the uninjured branch) in *E. flexuosa* [ANOVA, $F(3, 15) = 3.1$, $P = 0.060$] and *P. porosa* [ANOVA, $F(3, 14.1) = 2.7$, $P = 0.089$], although a trend existed whereby the protein content was lower in the injured than the uninjured branches (Fig. 2b). For both species, the areal content of host protein was significantly lower in tissues at and adjacent to the lesion than in the uninjured branches (Table 1). Conversely, carbohydrate content of the cortex in both species did not differ between the injured and uninjured branch regions (Table 1).

Melanin content and enzyme activity

Since sclerite, and thus organic content, significantly differed between the injured and uninjured branches, the effect of tissue injury on melanin content was only tested within sampled regions of the injured branches. In *E. flexuosa*, tissues at the lesion contained significantly less melanin in organic matter (%g OM) than the tissues surrounding and those located below the lesion (Table 1). Conversely, in *P. porosa,* melanin content did not significantly differ between the three regions of the injured branches (Table 1). The two species also exhibited contrasting trends in enzyme activity. PO and POX activities in *E. flexuosa* were significantly higher [PO: Fig. 2c, ANOVA, $F(3, 15) = 16.3$, $P < 0.001$; POX: Table 1], while in *P. porosa,* PO activity was significantly lower in tissues at and surrounding the lesion compared to tissues from below the lesion or from uninjured branches [Fig. 2c, ANOVA, $F(3, 14) = 12$, $P < 0.001$]. In *E. flexuosa,* EXOC activity was significantly lower in tissues at and surrounding the lesions compared to in tissues from below the lesions and from the uninjured branches [Fig. 2d, ANOVA, $F(3, 15) = 5.4$, $P = 0.010$]. In contrast, in *P. porosa*, EXOC activity did not significantly differ between injured and uninjured branches [Fig. 2d, ANOVA, $F(3, 14.1) = 1.4$, $P = 0.291$].

Symbiodinium **parameters**

Several of the *Symbiodinium* in this study were novel and ascribed to the new types B41a (GenBank Accession No. KX344964), B41b (GenBank accession no. KX344965), and B42 (GenBank Accession No. KX344981). Of the eight sampled *E. flexuosa* colonies, one hosted *Symbiodinium* type B41a, while seven contained B41b. In *P. porosa*, six colonies contained *Symbiodinium* type B1i (Finney et al. 2010; GenBank Accession No. GU907636), and two colonies contained type B42. Since different *Symbiodinium* exhibit different physiologies (Goulet et al. 2005; Ramsby et al. 2014), we excluded from the statistical analyses, the one and two colonies containing other *Symbiodinium* types. In both gorgonian species, the *Symbiodinium* type found in the healed tissues, the tissues surrounding the lesion site, and in the uninjured branches was the same.

In both *E. flexuosa* and *P. porosa*, *Symbiodinium* density (Fig. 3a, Paired *t* test, *E. flexuosa*: $t6 = 10.7$, $P < 0.001$; *P. porosa*: $t5 = 3.4$, $P = 0.020$) and Chl *a* (Fig. 3c, Paired *t* test, *E. flexuosa*: $t6 = 7.0$, $P < 0.001$; *P. porosa*: $t5 = 3.9$, $P = 0.012$) and c_2 (Table 2) per surface area were significantly less in tissues at and those surrounding the lesion

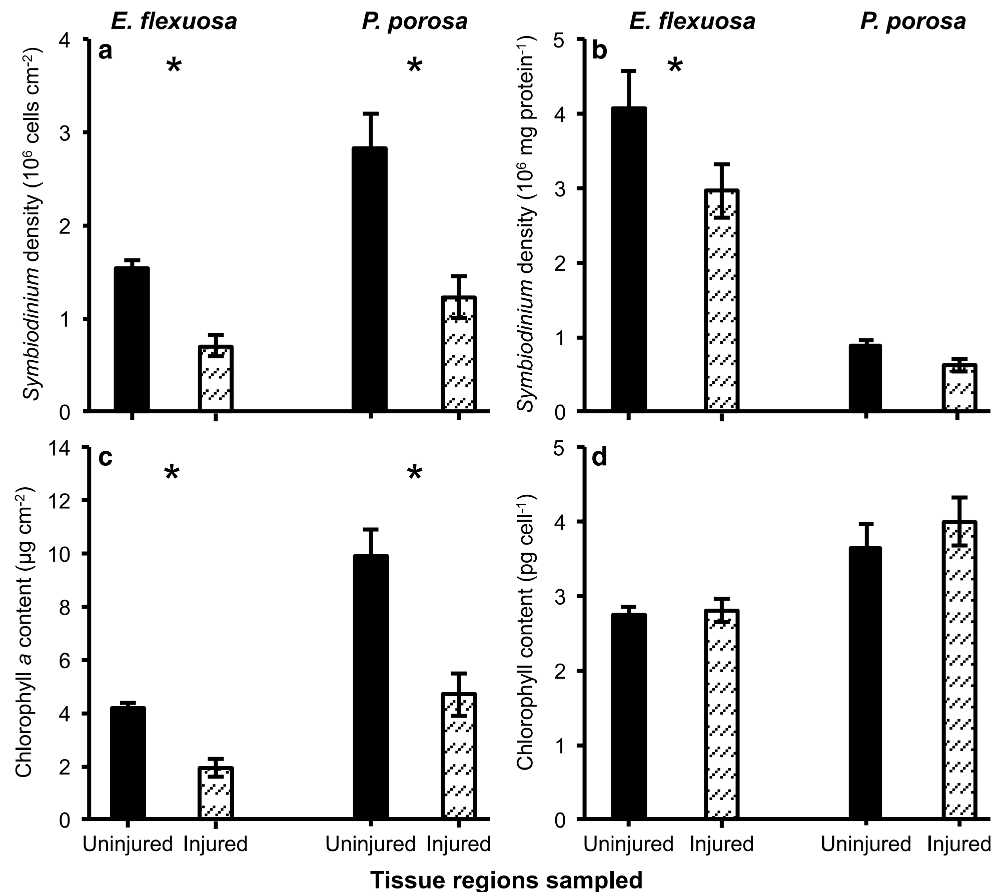

Fig. 3 Mean *Symbiodinium* density **a** per surface area and **b** per mg host protein, and chlorophyll *a* content, **c** per surface area and **d** per *Symbiodinium* cell, in the gorgonian corals *Eunicea flexuosa* and *Pseudoplexaura porosa*. Samples were taken from uninjured branches (Uninjured) and from areas in the injured branch containing the lesion on one side and uninjured tissue on the other (Injured). Data are mean ± SE, with $n = 7$ for *E. flexuosa* and $n = 6$ for *P. porosa*. Statistically significant results ($P < 0.05$) detected by paired *t* tests are denoted with (*asterisk*)

Table 2 Effect of injury on *Symbiodinium* parameters in the gorgonian corals *Eunicea flexuosa* and *Pseudoplexaura porosa*

Parameter	*E. flexuosa*				*P. porosa*			
	Uninjured	Injured	*t*6	*P*	Uninjured	Injured	*t*5	*P*
Chl *a* content (µg mg^{-1} protein)	11.10 ± 1.42	8.33 ± 1.16	4.7	**0.003**	3.11 ± 0.29	2.41 ± 0.30	2.5	0.058
Chl c_2 content (µg cm^{-2})	1.30 ± 0.10	0.62 ± 0.09	7.9	**<0.001**	3.01 ± 0.34	1.44 ± 0.22	3.6	**0.016**
Chl c_2 content (µg mg^{-1} protein)	3.44 ± 0.48	2.69 ± 0.35	4.9	**0.003**	0.93 ± 0.07	0.74 ± 0.08	2.2	0.084
Chl c_2 content (pg cell^{-1})	0.84 ± 0.05	0.92 ± 0.07	−1.3	0.245	1.08 ± 0.07	1.23 ± 0.09	−1.4	0.213
Chl *a*: c_2 ratio	3.31 ± 0.15	3.09 ± 0.11	1.2	0.276	3.32 ± 0.12	3.23 ± 0.09	1.4	0.227

Samples were processed from the uninjured branch (Uninjured) and the wounded region of the injured branch (Injured), which included tissue at the lesion and uninjured tissue on the other side of the branch. Values are mean ± SE ($n = 7$ for *E. flexuosa*, 6 for *P. porosa*) compared with paired *t* tests, where t = test statistic, P = probability of the null, <0.05 in bold. Chl = chlorophyll

than in the uninjured branches. Similar patterns occurred when symbiont density (Fig. 3b, Paired *t* test, *E. flexuosa*: $t6 = 4.0$, $P = 0.007$; *P. porosa*: $t5 = 2.0$, $P = 0.105$) and Chl *a* and c_2 contents (Table 2) were expressed per host protein, although in *P. porosa,* the difference was not statistically significant. In both species, the Chl *a* (Fig. 3d, Paired *t* test, *E. flexuosa*: $t6 = -0.4$, $P = 0.740$; *P. porosa*: $t5 = -0.9$, $P = 0.423$) and c_2 content per *Symbiodinium* cell and the ratio of Chl *a*: c_2 did not significantly differ between the lesion site, surrounding tissues, and the uninjured branches (Table 2).

F_v/F_m, $\Delta F/F_{m'}$, and Q_m did not significantly differ between the lower, middle, and upper regions of branches for either species. Therefore, the values from the three regions were pooled. During lesion recovery, F_v/F_m, $\Delta F/F_{m'}$, and Q_m in both gorgonian species did not significantly differ between tissues surrounding the lesion and those from the uninjured branches (Online Resources 1 and 2).

Bacterial community

The two gorgonian species harbored distinct bacterial communities, with 160,736 (mean: 8113, median: 7789) and 137,924 (mean: 9455, median: 5063) bacterial sequences isolated from *E. flexuosa* and *P. porosa,* respectively. Bacterial sequence data are available in the NCBI Sequence Reads Archive under accession SRP076113. In uninjured branches of *E. flexuosa,* the majority (73 %) of bacterial sequences could not be classified, while Proteobacteria comprised 21 % of the sequences (Fig. 4a; Table 3). In *P. porosa*, the phyla Tenericutes (77 %) and Proteobacteria (15 %) accounted for most of the bacterial community in uninjured branches (Fig. 4a; Table 3). In both gorgonian species, other phyla individually comprised <2 % of the bacterial community (Online Resource 3).

The prevalence of Tenericutes in *P. porosa*, and Proteobacteria in *E. flexuosa*, did not differ between injured and uninjured branches (Fig. 4a; Table 3). In contrast, Proteobacteria in *P. porosa* were significantly less prevalent at the lesion and adjacent tissues than in the uninjured branch (Fig. 4a; Table 3). Within Proteobacteria, in both gorgonian species, the class Gammaproteobacteria (Table 3), specifically the family Hahellaceae (which in the literature sometimes appears as Endozoicimonaceae, Neave et al. 2016), were significantly less prevalent in tissues at and surrounding the lesion, than in uninjured branches [Fig. 4b, ANOVA, *E.* flexuosa: $F(2, 8) = 20$, $P < 0.001$; *P. porosa*: $F(2, 9) = 18.2$, $P < 0.001$]. Furthermore, Hahellaceae in *E. flexuosa* were significantly less prevalent in tissues at than in those surrounding the lesion (Fig. 4b). In contrast, the family Enterobacteriaceae in both gorgonian species

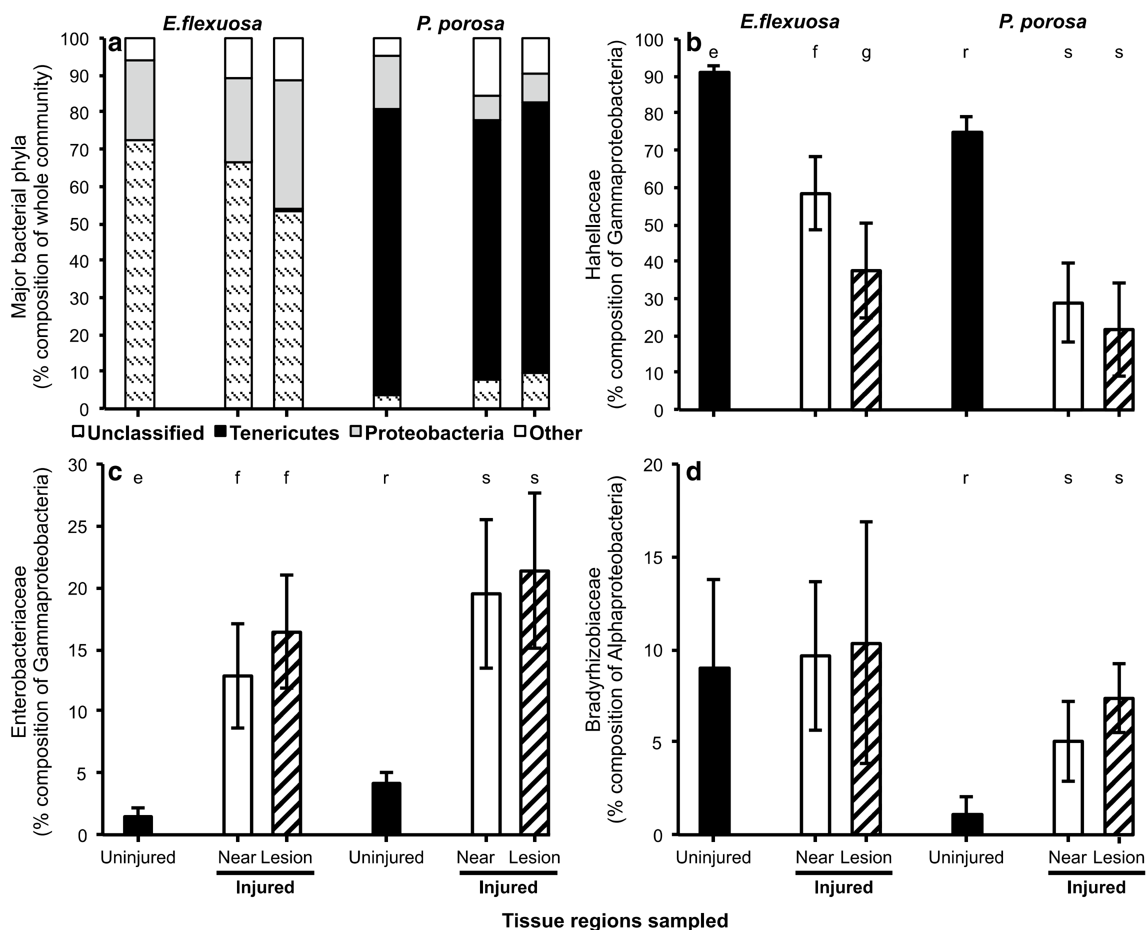

Fig. 4 Prevalence (%) of major bacterial phyla within the microbiome (**a**), bacterial families Hahellaceae (**b**) and Enterobacteriaceae (**c**) within Gammaproteobacteria, and the family Bradyrhizobiaceae (**d**) within Alphaproteobacteria, in tissues of the gorgonian corals *Eunicea flexuosa* and *Pseudoplexaura porosa*. Samples were taken from uninjured branches (Uninjured) and two regions of injured branches (Injured): uninjured tissue adjoining the lesion (Near), and regenerated tissue at the lesion (Lesion). Data are presented as mean ± SE, with $n = 5$ for most parameters and $n = 6$ for *E. flexuosa*-Lesion and *P. porosa*-Near and Lesion in (**c**), and *E. flexuosa*-Near and *P. porosa*-Lesion in (**d**). *Letters* (*e–g* for *E. flexuosa* and *r*, *s* for *P. porosa*) above the *bars* denote significant differences ($P < 0.05$) in the planned contrasts

Table 3 Effect of injury on the prevalence (%) of major bacterial phyla and Proteobacteria subclasses in the gorgonian corals *Eunicea flexuosa* and *Pseudoplexaura porosa*

Bacterial taxa	Uninjured	Injured		$F(\mu, \nu)$	P
		Near	Lesion		
E. flexuosa					
Tenericutes	0 ± 0	$0.006^{¥} \pm 0.009$	0.004 ± 0.004	NA	
Proteobacteria	21.06 ± 4.28	$22.78^{¥} \pm 7.97$	$35.20^{¥} \pm 6.86$	$F(2, 9.2) = 2.0$	0.185
Gammaproteobacteria	$19.11^{e} \pm 4.10$	$6.34^{f} \pm 2.35$	$6.11^{f} \pm 2.01$	$F(2, 8.0) = 5.2$	**0.023**
Pseudomonadaceae^	$3.45^{e} \pm 1.14$	$8.77^{¥e} \pm 2.39$	$17.08^{¥f} \pm 7.11$	$F(2, 9.1) = 4.8$	**0.038**
Alphaproteobacteria^	$1.12^{e} \pm 0.19$	$2.03^{e} \pm 0.72$	$21.14^{¥f} \pm 5.99$	$F(2, 8.7) = 29.5$	**<0.001**
Rhodobacteraceae+	14.68 ± 6.50	3.92 ± 1.22	$36.15^{¥} \pm 14.20$	$F(2, 8.9) = 2.7$	0.123
Methylobacteriaceae	8.26 ± 4.87	$13.72^{¥} \pm 3.98$	7.16 ± 3.62	$F(2, 8.8) = 0.9$	0.455
Hyphomicrobiaceae	6.73 ± 3.24	9.91 ± 3.25	7.16 ± 2.08	$F(2, 8.3) = 0.3$	0.727
Unclassified	72.69 ± 5.23	$66.45^{¥} \pm 10.20$	$53.52^{¥} \pm 7.85$	NA	
P. porosa					
Tenericutes+	76.91 ± 5.60	$70.48^{¥} \pm 9.07$	$72.85^{¥} \pm 6.78$	$F(2, 9.3) = 0.1$	0.878
Proteobacteria+	$14.51^{r} \pm 3.51$	$6.63^{\$s} \pm 0.91$	$7.60^{s} \pm 1.53$	$F(2, 7.2) = 5.7$	**0.032**
Gammaproteobacteria^	$6.71^{r} \pm 1.80$	$2.13^{s} \pm 0.18$	$2.31^{s} \pm 0.60$	$F(2, 8.0) = 7.3$	**0.016**
Pseudomonadaceae	$5.13^{r} \pm 1.22$	$22.82^{¥s} \pm 7.81$	$22.31^{¥s} \pm 7.60$	$F(2, 9.1) = 6.6$	**0.017**
Alphaproteobacteria^	5.13 ± 1.76	3.70 ± 0.53	$4.57^{¥} \pm 0.72$	$F(2, 8.2) = 1.5$	0.275
Rhodobacteraceae	2.62 ± 0.59	$2.86^{¥} \pm 1.16$	$2.33^{¥} \pm 0.73$	$F(2, 9.2) = 0.2$	0.847
Methylobacteriaceae^	3.71 ± 1.96	18.46 ± 6.72	$19.27^{¥} \pm 8.61$	$F(2, 9.2) = 2.7$	0.117
Hyphomicrobiaceae^	2.19 ± 1.76	$4.93^{¥} \pm 2.40$	$2.09^{¥} \pm 0.91$	$F(2, 9.5) = 0.6$	0.563
Unclassified	3.90 ± 1.69	$7.61^{¥} \pm 3.11$	$9.88^{¥} \pm 4.04$	NA	

The prevalence of families is presented as the relative abundance within Gammaproteobacteria and Alphaproteobacteria. The prevalence of sequences that could not be classified into a phylum was not analyzed. Bacteria were identified from the uninjured branch (Uninjured) and two regions in the injured branch (Injured): tissue adjacent to the lesion on the other side of the branch (Near), and regenerated tissue at the lesion (Lesion). Only those phyla that individually accounted for ≥3 % of the total bacterial community are shown. Values are mean ± SE with $n = 5$, $n = 6$ (¥) or $n = 4$ ($). $F(\mu, \nu) = F$-ratio and degrees of freedom, $P =$ probability of the null, <0.05 in bold. To meet normality and heteroscedasticity assumptions, some parameters were log (^) or arcsine square root (+) transformed. Superscript letters (e, f for *E. flexuosa*, r, s for *P. porosa*) denote significant differences ($P < 0.05$) in the planned contrasts

[Fig. 4c, ANOVA, *E. flexuosa*: $F(2, 8.3) = 11.1$, $P = 0.005$; *P. porosa*: $F(2, 9.1) = 8.5$, $P = 0.008$] and Pseudomonadaceae in *P. porosa* (Table 3) were significantly more prevalent in injured than in uninjured branches. Pseudomonadaceae and the class Alphaproteobacteria in *E. flexuosa* were significantly more prevalent in tissues at than in tissues surrounding the lesion and from uninjured branches (Table 3). Within the Alphaproteobacteria, in both gorgonian corals, the prevalence of families Rhodobacteraceae, Methylobacteriaceae, and Hyphomicrobiaceae did not significantly differ between injured and uninjured branches (Table 3). In *P. porosa*, the family Bradyrhizobiaceae was significantly more prevalent in injured than in uninjured branches [Fig. 4d, ANOVA, *E. flexuosa*: $F(2, 9.2) = 0.1$, $P = 0.895$; *P. porosa*: $F(2, 8.9) = 4.4$, $P = 0.047$].

At a finer taxonomic resolution 1818, OTUs were identified and a significantly different bacterial consortium was found associated with each species [Fig. 5, AMOVA, $F(1, 33) = 82$, $P < 0.001$]. Certain OTUs occurred in one gorgonian species and not the other (Fig. 5), while others were

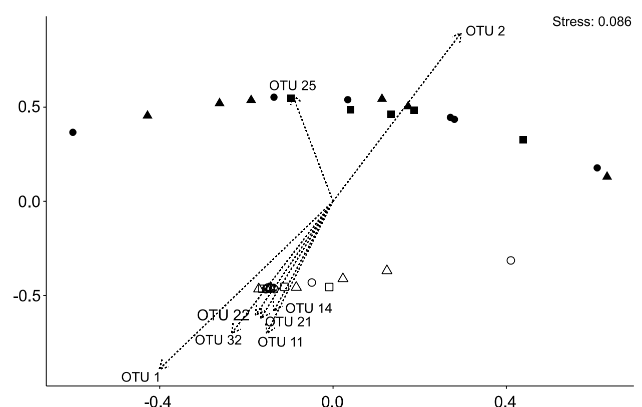

Fig. 5 Non-metric multidimensional scaling ordination plot generated using the OTUs identified in the gorgonian corals *Eunicea flexuosa* (*filled symbols*) and *Pseudoplexaura porosa* (*open symbols*). Samples were taken from uninjured branches (*squares*) and two regions of injured branches: uninjured tissue adjoining the lesion (*circles*), and regenerated tissue at the lesion (*triangles*). *Dotted arrows* indicate OTUs that primarily associated with one or the other gorgonian species

Table 4 Effect of injury on the total number, and prevalence (% total community), of OTUs in the bacterial microbiome of the gorgonian corals *Eunicea flexuosa* and *Pseudoplexaura porosa*

OTU	Uninjured	Injured		$F(\mu, \nu)$	P
		Near	Lesion		
E. flexuosa					
Total OTUs	$32.58^{\#e} \pm 4.05$	$35.09^{e} \pm 6.79$	$54.99^{f} \pm 7.35$	$\chi^2 = 40.92$, $P <$ **0.001**	
1: g_*Mycoplasma* sp.	$0^{\#} \pm 0$	0.006 ± 0.004	0 ± 0	NA	
2: p_Unclassified	$46.34^{\#} \pm 6.12$	39.68 ± 7.88	36.94 ± 7.96	$F(2, 9.5) = 0.4$	0.697
3: p_Unclassified	$25.81^{\#} \pm 7.93$	26.18 ± 10.63	15.90 ± 7.99	$F(2, 9.1) = 1.2$	0.335
P. porosa					
Total OTUs	$35.65^{\#} \pm 3.97$	39.37 ± 4.97	35.93 ± 4.67	$\chi^2 = 1.28$, $P < 0.526$	
1^{+}: g_*Mycoplasma* sp.	$77.15^{\#} \pm 5.58$	70.69 ± 9.10	73.00 ± 6.79	$F(2, 9.3) = 0.1$	0.877
2: p_Unclassified	$0^{\#} \pm 0$	0 ± 0	0 ± 0	NA	
3^{+}: p_Unclassified	$2.12^{\#} \pm 1.32$	7.30 ± 3.17	9.68 ± 4.08	$F(2, 9.1) = 3.7$	0.067

Bacteria were identified from the uninjured branch (Uninjured) and two regions in the injured branch (Injured): tissue adjacent to the lesion on the other side of the branch (Near), and regenerated tissue at the lesion (Lesion). The three most abundant OTUs in the bacterial community are listed. Sequence reads were normalized to 425 OTUs per sample. Values are mean ± SE, with $n = 6$ or $n = 5$ (#). $F(\mu, \nu) = F$-ratio and degrees of freedom, P = probability of the null, <0.05 in bold. Differences in total number of OTUs were tested using a generalized linear model, where X = Chi-square test statistic and P = probability of the null, <0.05 in bold. To meet normality and heteroscedasticity assumptions, some parameters were arcsine square root (+) transformed. Superscripts (e, f for *E. flexuosa*) denote significant differences ($P < 0.05$) in the planned contrasts. Prefixes denote p = phylum, g = genus

found in both species (Table 4). In both gorgonian species, the bacterial community structure [AMOVA, *E. flexuosa*: $F(2, 16) = 0.4$, $P = 0.813$; *P. porosa*: $F(2, 16) = 0.4$, $P = 0.760$] and the prevalence of dominant OTUs 2 and 3 (Unclassified bacteria) in *E. flexuosa*, and OTU 1 (*Mycoplasma* sp.) and OTU 3 in *P. porosa,* did not significantly differ between injured and uninjured branches (Table 4). In *E. flexuosa*, tissues at the lesion site contained significantly more OTUs than tissues surrounding the lesion and in the uninjured branches, while in *P. porosa*, the number of OTUs did not significantly vary between sampled tissues (Table 4).

Discussion

Gorgonian corals dominate many Caribbean coral reefs (Goldberg 1973; Kinzie III 1973; Jordán-Dahlgren 1989; Ruzicka et al. 2013), and how these corals respond to injury may help explain their abundance. Wound healing can vary based on lesion characteristics (Cary 1914; Lang da Silveira and Van't Hof 1977; Meesters et al. 1997; Oren et al. 1997; van Woesik 1998) and environmental conditions (Wahle 1983; Henry and Hart 2005; Denis et al. 2011; Sabine et al. 2015). Since in our study, these variables were similar for both *E. flexuosa* and *P. porosa*, the different recovery rates likely reflect interspecies variability in recovery similar to the observed ranges of 6–8 days in *E. mammosa*, 7–9 days in *Plexaura homomalla*, and 7–11 days in *Plexaurella dichotoma* (Wahle 1983). Furthermore, although *E. flexuosa* and *P. porosa* both belong to the family Plexauridae (Wirshing et al. 2005; Daly et al. 2007), they may be quite different from one another. In chemotaxonomic and phylogenetic studies, *Eunicea* spp. clustered separately from *Pseudoplexaura* spp., but since the techniques used could only resolve clade-level differences, the clusters were not significantly different (Gerhart 1983; Sánchez et al. 2003; Wirshing et al. 2005). In another study, on the phylogenetic relationships between *Eunicea* species, where finer resolution was possible with the ITS2 marker, *Pseudoplexaura crucis*, which is in the same genus as *P. porosa* (Wirshing et al. 2005), was used as an outgroup, demonstrating the differences between *Eunicea* spp. and *Pseudoplexaura* spp. (Grajales et al. 2007). Therefore, taxonomic separation may contribute to the differences between *E. flexuosa* and *P. porosa* including why lesion recovery in *E. flexuosa* took twice as long as in *P. porosa,* although recovery in *E. flexuosa* was comparable to an earlier study on that species (Lang da Silveira and Van't Hof 1977).

Energetic sources for lesion recovery

E. flexuosa and *P. porosa* also differ in their biochemical composition. The combined sclerite and refractory content in *E. flexuosa* (81 and 13 % of dry weight) is higher than that in *P. porosa* (46 % sclerite and 29 % refractory content per dry weight) (Shirur et al. 2014). Consequently, protein, lipid, and carbohydrate content together average just 6 % of the dry weight of *E. flexuosa* compared to over four times as much (25 %) in *P. porosa* (Shirur et al. 2014). Using the

mean protein, lipid and carbohydrate content per organic matter (Shirur et al. 2014), and their enthalpies of combustion (Gnaiger and Bitterlich 1984), we estimate that tissues in *E. flexuosa* contain almost 40 % less energy than those in *P. porosa* (9650 vs. 15,730 J g^{-1} OM). Furthermore, *E. flexuosa* polyps capture fewer and smaller prey than *P. porosa* polyps (Ribes et al. 1998).

In addition, *Symbiodinium* photosynthesis in *E. flexuosa* may be lower than that in *P. porosa.* The net photosynthetic rate of *Symbiodinium* in *E. tourneforti*, a species with a biochemical composition similar to *E. flexuosa* (Shirur et al. 2014), is almost three times lower than in *P. porosa* (Ramsby et al. 2014). This could be due to genetic differences between the gorgonians (Gerhart 1983; Wirshing et al. 2005; Grajales et al. 2007) as well as different *Symbiodinium* types (Ramsby et al. 2014). Besides photosynthetic differences at ambient conditions, biochemical changes in branches during lesion recovery could affect *Symbiodinium* in surrounding tissues. Diversion of organic molecules by the host toward lesion recovery could reduce the host's regular translocation of inorganic carbon, fatty acids, and nitrogenous compounds to the *Symbiodinium* (Hughes et al. 2010; Imbs et al. 2014; Tanaka et al. 2015), subsequently affecting photosynthesis and the routine transfer of photosynthetic products to the host (Kopp et al. 2015). *Symbiodinium* in tissues near the lesion, however, did not seem to be affected since their photochemical parameters, chlorophyll content of their cells, and the Chl a:c_2 ratio did not differ from those in the uninjured branches. Similarly, in the scleractinian corals *Pocillipora verrucosa* and *Acropora muricata,* the maximum quantum yield of photosystem II did not differ between tissues surrounding a lesion and those in uninjured branches (Lenihan and Edmunds 2010; Denis et al. 2013).

Conversely, *Symbiodinium* density was lower in tissues near the lesion than in uninjured branches. In scleractinian corals, *Symbiodinium* density depends on the host biomass available to harbor the algae (Drew 1972; Jones and Yellowlees 1997; Thornhill et al. 2012). In our study, in both gorgonian corals, sclerite content was significantly higher and host protein content per surface area was significantly lower at the lesion and in surrounding tissues than in uninjured branches, indicating that the regions near the lesion contained less host biomass. Therefore, fewer *Symbiodinium*-containing host cells in the injured gorgonian branches may have led to the lower *Symbiodinium* density. Alternatively, lower *Symbiodinium* density could occur because the remaining host cells each contained fewer *Symbiodinium*. *Symbiodinium* density per host protein was lower in injured branches of *E. flexuosa* and *P. porosa*, although this difference was significant only in *E. flexuosa*. Thus, the longer recovery time in *E. flexuosa* compared to *P. porosa* may reflect a combination of less protein, lipids, and carbohydrates and hence existing energy reserves, and lower amounts of heterotrophically and photosynthetically acquired nutrients.

The process of lesion recovery and its implications

Although the rate of recovery differed between the two species, sclerites were visible at the lesion sites in *E. flexuosa* and *P. porosa,* and sclerite content in both species was significantly higher at the lesion and in surrounding tissues than in uninjured branches. This suggests that sclerites are integral to lesion recovery. Consequently, conditions that detrimentally affect sclerites may adversely affect wound healing. For example, in scleractinian corals, low pH hinders calcification (Hoegh-Guldberg et al. 2007; Anthony et al. 2011; Dove et al. 2013). Potentially, because sclerites are embedded within tissues, low pH conditions did not affect sclerites in the gorgonian corals *E. flexuosa* (Enochs et al. 2016), *E. fusca* (Gómez et al. 2015) and in the octocoral *Ovabunda macrospiculata* (Gabay et al. 2014). The lack of effect on sclerites could be one of the reasons for a transition from scleractinian coral to octocoral-dominated benthos closer to naturally occurring volcanically acidified water (Inoue et al. 2013). On the other hand, when sclerites were removed from tissues of the octocoral *O. macrospiculata,* thereby directly exposing them to lower pH, partial sclerite dissolution did occur (Gabay et al. 2014). Ocean acidification could therefore detrimentally affect exposed sclerites at lesion sites and hinder wound healing in gorgonian corals, potentially, similar to the effect of lower pH on wound healing in scleractinian corals (Horwitz and Fine 2014; Hall et al. 2015).

The bacterial community in injured and uninjured tissues

In addition to hosting *Symbiodinium* spp. (zooxanthellae), scleractinian corals and octocorals contain a consortium of organisms and their microbiome (Knowlton and Rohwer 2003; Toledo-Hernández et al. 2008; Sunagawa et al. 2010; Hewson et al. 2011; Thompson et al. 2015). Since the bacterial portion of this microbiome was unknown for *E. flexuosa* and *P. porosa*, we characterized these communities. In *E. flexuosa,* the majority of bacteria were identified as two OTUs (OTU 2, 3) that could be classified only to Bacteria, while in *P. porosa*, the majority of bacteria belonged to Tenericutes (OTU 1: *Mycoplasma* spp.). Tenericutes are host-specific commensals or parasites (Razin et al. 1998) which can be major components of the bacterial microbome in azooxanthellate octocorals (Gray et al. 2011; Porporato et al. 2013; Holm and Heidelberg 2016) and the azooxanthellate scleractinian coral *Lophelia pertusa* (Kellogg et al. 2009). They are rare or absent in zooxanthellate

gorgonian and scleractinian corals in which Proteobacteria dominate the bacterial community (Sunagawa et al. 2010; Cárdenas et al. 2012; Duque-Alarcón et al. 2012; Lee et al. 2012; Correa et al. 2013; Tracy et al. 2015; McCauley et al. 2016; Robertson et al. 2016).

Proteobacteria was the second most abundant phylum in both *E. flexuosa* and *P. porosa*, represented predominantly by the Gammaproteobacteria and Alphaproteobacteria classes. Among Gammaproteobacteria, most were Hahellaceae, a group that may be part of the core microbiome of scleractinian corals and octocorals (Bayer et al. 2013; Correa et al. 2013; La Rivière et al. 2013, 2015; McCauley et al. 2016; Neave et al. 2016; Robertson et al. 2016). The Hahellaceae in our samples were most closely related to *Endozoicomonas euniceicola* and *E. gorgoniicola* identified from the Caribbean gorgonian corals *Eunicea fusca* and *Plexaura* sp., respectively (Pike et al. 2013), *E. montiporae* and *E. elysicola* isolated from the scleractinian coral *Montipora aequituberculata* (Yang et al. 2010) and the slug *Elysia ornata* (Kurahashi and Yokota 2007), respectively, from the Indo-Pacific.

Other Gammaproteobacteria were identified as Pseudomonadaceae, which have been previously found in octocorals and scleractinian corals (Penn et al. 2006; Duque-Alarcón et al. 2012; Lee et al. 2012; Morrow et al. 2012; Bourne et al. 2013; Correa et al. 2013). Lastly, 1.5 % of Gammaproteobacteria in *E. flexuosa* and 4.2 % in *P. porosa* were Enterobacteriaceae, including *Escherichia coli*. The *E. coli* source could be runoff from housing along the coastline, or from contaminated groundwater (Baker et al. 2010; Leal-Bautista et al. 2011), which around the town Puerto Morelos may reach the ocean through underwater springs (Carruthers et al. 2005). Among the Alphaproteobacteria, two orders, Rhodobacterales (primarily Rhodobacteraceae) and Rhizobiales (Methylobacteriaceae, Hyphomicrobiaceae and Bradyrhizobiaceae), were the most prevalent, both of which have been reported in octocorals and scleractinian corals (Penn et al. 2006; Kellogg et al. 2009; Mouchka et al. 2010; Gray et al. 2011; Duque-Alarcón et al. 2012; Morrow et al. 2012; Bourne et al. 2013; Lema et al. 2014; Ainsworth et al. 2015; Robertson et al. 2016). In *E. flexuosa* and *P. porosa*, 9 and 73 % of the Alphaproteobacteria could not be classified further (Online Resource 4).

Injury did not cause a change in the overall structure of the bacterial microbiome of *E. flexuosa* and *P. porosa*, a finding similar to that in the scleractinian coral *Acropora aspera* (van de Water et al. 2015). The prevalence of some individual bacterial taxa did differ between injured and uninjured branches. Hahellaceae were 2.4× and 1.6× less prevalent in tissues at and adjacent to the lesion in *E. flexuosa* than in uninjured branches, and in injured branches of *P. porosa*, they were 3× less prevalent. A reduction in the prevalence of Hahellaceae also occurred in corals sampled from sites exposed to human impacts (Vezzulli et al. 2013; Roder et al. 2015; Ziegler et al. 2016), acidified conditions (Morrow et al. 2015), and in corals exhibiting bleaching (Bourne et al. 2008) or tissue lesions (Meyer et al. 2014).

In contrast, the proportion of *E. coli* was significantly higher (9.8× for *E. flexuosa*, 4.8× for *P. porosa*) at sites at and surrounding the lesion. A similar pattern presented itself for Pseudomonadaceae in *E. flexuosa* at the lesion site (2.8×) and in *P. porosa* (4.4×) in tissues at and surrounding the lesion. Increases in the prevalence of Pseudomonadaceae and Rhizobiales also occurred in white plague diseased tissues of scleractinian corals, but these bacteria may be opportunistic rather than the disease-causing pathogens (Sunagawa et al. 2009; Mouchka et al. 2010; Cárdenas et al. 2012; Roder et al. 2014). In addition, in *E. flexuosa*, the injured sites had a more diverse range of bacterial OTUs (1.6×). In *P. porosa*, Bradyrhizobiaceae were more prevalent (5.4×) near and at the lesion site, and injury-induced shifts also occurred in some less prevalent (≤1 % of the bacterial community) Gammaproteobacteria and Alphaproteobacteria taxa (Online Resources 4, 5). Never the less, given that tissue recovery proceeded in both *E. flexuosa* and *P. porosa* without symptoms of disease, the two species coped with the lesions and the bacteria present in the injured area, although their coping mechanisms may differ.

Defense mechanisms against pathogens

In *E. flexuosa,* even though some bacterial groups were more abundant at the lesion than in surrounding tissues, the lack of a major bacterial community shift could have been due to immune activity. Defensive mechanisms such as the nonspecific immune responses melanin and PO may protect corals against pathogens (Toledo-Hernández and Ruiz-Diaz 2014). In *E. flexuosa*, the lesion site and the surrounding tissues had lower melanin content and higher PO and POX activity compared to levels and activity away from the lesion, indicating that the melanization cascade was activated. Melanin may have been released from cells to aid in clot formation as seen in the scleractinian coral *Porites cylindrica* (Palmer et al. 2011b), or to coat pathogens to limit their proliferation at the lesion as in the gorgonian *G. ventalina* (Petes et al. 2003; Mydlarz et al. 2008). In addition, high PO activity may produce additional melanin, and intermediate reactive oxygen species (ROS) and quinones that are cytotoxic to pathogens (Cerenius and Söderhäll 2004; Cerenius et al. 2008). High POX activity may protect tissues from ROS produced by the action of PO (Mydlarz and Palmer 2011), and aid in preventing fungal infection at the lesion site (Mydlarz and Harvell 2007). While melanin and PO are nonspecific immune responses against pathogens in general, EXOC

specifically breaks down fungal cell walls. In *E. flexuosa,* EXOC activity was significantly lower at the lesion and in surrounding tissues than in tissues away from the lesion, and therefore, as in *G. ventalina*, EXOC may have been released from tissues to attack fungi in the water surrounding the lesion (Douglas et al. 2007).

Similar to *E. flexuosa,* even though in *P. porosa* the proportion of some minor components of the bacterial microbiome were different in injured versus uninjured branches, the overall bacterial community did not change. In contrast to *E. flexuosa, P. porosa* did not exhibit activation of many parameters associated with an immune response. Melanin content did not vary, and PO activity was significantly lower in tissues at and surrounding the lesion than further away, suggesting that fewer components of the melanization cascade were activated. Furthermore, EXOC activity did not differ between sampled tissues. Therefore, *P. porosa* may employ alternate defense mechanisms (Perkins and Ciereszko 1973; Rodríguez 1995; Jensen et al. 1996; Hunt et al. 2012). Tissue extracts of gorgonian species, including *E. flexuosa* and *P. porosa*, contain substances with antimicrobial activity (Jensen et al. 1996). In addition, gorgonian compounds may alter quorum sensing between bacteria (Hunt et al. 2012) which could affect bacterial virulence (Waters and Bassler 2005; Rutherford and Bassler 2012), although in gorgonian corals, the mechanism of these defenses is unknown. The composition of such substances may be different and/or the amount may be higher in *P. porosa* than in *E. flexuosa*. For example, *P. porosa* tissue extracts are significantly more potent against the fungal pathogen *Aspergillus sydowii* than those from *E. flexuosa* (Kim et al. 2000), and *P. porosa* tissues contain crassin acetate, which is toxic to ciliates (Perkins and Ciereszko 1973).

Conclusion

The Caribbean gorgonian corals *E. flexuosa* and *P. porosa* dealt with artificially induced lesions, and the wounds healed with no visible signs of infection or necrosis. The two gorgonian species, however, differed in how they coped with the lesions, probably due to the structural, biochemical, and symbiotic differences between the two. *E. flexuosa* and *P. porosa* also differed in their ambient bacterial consortia and in their immune response. These multiple distinctions illustrate the importance of investigating numerous gorgonian species. Despite their differences, both *P. porosa* and *E. flexuosa* handled the potential stress brought about by injury. The capacity to recover from this stressor may in part explain why gorgonian corals are maintaining and/or increasing in abundance on Caribbean reefs.

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Compliance with ethical standards

Conflict of interest The authors have no conflict of interest. Gorgonian corals were sampled with a collection permit.

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